



STATUS REPORT ON THE WORK OF MARINALG INTERNATIONAL TO MEASURE THE MOLECULAR WEIGHT DISTRIBUTION OF CARRAGEENAN AND PES IN ORDER TO MEET THE EU SPECIFICATION: LESS THAN 5% BELOW 50,000 DALTONS

SUMMARY (as of January 2012)

In 2004 the European Commission published new specifications for carrageenan and PES requiring that carrageenan and PES for used in food must not contain more than 5% molar mass with molecular weight less than 50,000 Da. Since then Marinalg has been working on developing a reliable analytical method for determining the low molecular weight tail (LMT) of carrageenan and PES.

After eight years of planning, experimentation, and analysis (2003 to 2011), Marinalg has been unable to reliably measure this new specification in the laboratories of its members, its customers, or in independent laboratories. However, of greater significance to consumer safety, are the results of a recent 90-day rat feeding study that has shown no adverse toxicological effect of carrageenan in any of the tests animals. The carrageenan used in this feeding study was selected to contain a high molar mass less than 50,000 Da. it had a water viscosity of 8cps just barely above the 5cps limit. As a rough estimate it contained 6% to 10% carrageenan less than 50,000 Da. This work has now been published and appears in its entirety as Annex III of this report (Weiner, *et al*).

Marinalg has tested several methods based on size exclusion chromatography (SEC) and light scattering (LS), and none gave reliable and reproducible results. Below 50,000 Daltons, the signal to noise ratio when light scattering is used for molecular weight determination is too low for the accuracy required by the EU specification. A very expensive embodiment of SEC/LS has been adopted by the Japan Ministry of Health for measuring this specification, but the methodology is flawed, partly because pullulan standards were used to calibrate the SEC columns (Uno, *et al*, Annex VIII). Pullulan is not a good model for carrageenan for this purpose because the two polymers have different molecular shapes in solution, carrageenan being the more linear of the two.

To correct this deficiency, ten carrageenan molecular weight standards were prepared for the molecular weight (Mw) range of 600k Da to 12k Da. The polydispersity indexes (PDI) of the Mw standards between 600k Da and 73k Da were all less than 2.0 (1.3 - 1.8) indicating fairly sharp profiles. The PDI values of the lower molecular weight standards (56k Da to 12k Da) are in the range 3.5 – 5.0, and

too polydisperse for column calibration. The eight pullulan standards covering the same molecular weight range had PDI values less than 1.3. Further work is being considered to prepare carrageenan standards with lower PDI values, but this is a significant hurdle that will be difficult to achieve.

Another technology has been explored to try to achieve the desired molecular separation. This is membrane or ultrafiltration separation. The results using membranes with manufacturers' reported porosities of 30k Da and 100k Da clearly indicate that higher porosity membranes in the nominal range of 200-400k Da would be necessary to be permeable to the specified 50k Da carrageenan molecules. This will require a significant advancement in membrane manufacturing. No further work is planned using ultrafiltration at this time.

The Marinalg Working Group plans to re-examine its work on SEC/LS methods and some recent work of other scientists (e.g. Uno, Annex VIII, Spichtig, Annex VII) to determine if any opportunities for improvement have been overlooked. Certainly SEC/LS has many practical attributes that makes it desirable for the present use. However, new ideas for increasing the probability of successfully preparing standards in adequate quantity to be adopted for routine testing are needed. There is little justification for investing a lot of money on method development unless there is a high probability that fundamentals like baseline drift and signal to noise in the low molecular weight tail can be improved.

Marinalg is reaching a stage in this work where its findings should be discussed with EFSA or DG Sanco. An opportunity will present itself between now and 2016 when EFSA will be re-evaluating the specifications of carrageenan and PES. Preliminary results, particularly the feeding study noted above have been provided to JECFA as part of its 2007 review of carrageenan. As of the date of this report JECFA has not seen it necessary to apply the molecular weight distribution specification. The same is true for the US Food Chemical Codex after their extensive review of the carrageenan monograph in 2008.

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